Quantifying clearance rates of restored shellfish reefs using modular baskets

Maja Andersson[1,2], Karen L. Cheney[3,4], Robbie Porter[5], Ben L. Gilby[6], Benjamin Mos[4,7]

[1] School of the Environment, The University of Queensland, Brisbane, QLD, 4072 Australia

[2] Biology Education Centre, Uppsala University, Norbyvägen 14, 752 36 Uppsala, Sweden

[3] Marine Sensory Ecology Group, School of the Environment, The University of Queensland, Brisbane, QLD, 4072 Australia

[4] Centre for Marine Science, The University of Queensland, Brisbane QLD, 4072 Australia

[5] OzFish Unlimited Ltd, Level 1, 299 River Street, Ballina, NSW, 2470, Australia

[6] School of Science, Technology and Engineering, University of the Sunshine Coast, Petrie, QLD, 4502, Australia

[7] Moreton Bay Research Station, School of the Environment, The University of Queensland, Dunwich/Goompi, QLD, 4183, Australia

Corresponding author: B. Mos

Address: Moreton Bay Research Station, School of the Environment, The University of Queensland, 37 Fraser Street, Dunwich, QLD 4183, Australia

Email: b.mos@uq.edu.au

Keywords: oyster reef, restoration, filtration, clearance rate, bivalve, climate change, marine heatwave, flood

Author Contribution Statement: K.C., R.P., B.M. conceived the project. B.M., M.A., K.C., B.G. designed the experiments. B.M., R.P., K.C. provided resources. M.A., B.M. built the seawater system and ran the experiments. M.A. collated and graphed the data. B.M., M.A. analysed the data. M.A. wrote the manuscript. B.M., K.C., R.P., and B.G. reviewed and edited the manuscript.

Abstract

Shellfish reefs are one of the most degraded marine ecosystems, prompting substantial efforts to restore them. While biodiversity gains of restored reefs are well documented, other ecosystem services such as water filtration remain overlooked. We tested whether modular baskets could provide a practical way to measure water filtration by invertebrate communities on restored reefs and assess community responses to light, a simulated heatwave, and a simulated flood. A seawater system was designed to host ten modular baskets that had been deployed intertidally for 19 months in Moreton Bay, Australia. We measured baseline clearance rates and then tested the effects of (1) light by covering tanks with black polyethylene, (2) temperature by heating half of the tanks ~4 °C above ambient for five days, and (3) reduced salinity by addition of freshwater from ambient (~36) to ~25 or ~15 respectively. Microalgae *Nannochloropsis oceanica* (CS-246) was added at a density of 1–1.5 × 10^6 cells mL^{-1}, and algal density was measured every 30 min for up to 2 h. Mean baseline clearance rates per basket were 119.1 L h^{-1} ± 14.8 SE. Clearance rates were reduced by ~45% when the salinity was ~15 compared to ~25 but were not affected by light (light *vs* dark) or temperature (ambient *vs* +4 °C). Our results demonstrate modular restoration structures can be used to quantify the ecosystem services provided by restored reefs and to assess the vulnerability of natural and restored shellfish communities to current and future threats such as heatwaves and floods.

Introduction

Shellfish reefs form when living bivalves aggregate on hard substrates in subtidal and intertidal areas (Kennedy and Sanford 1999; Beck et al. 2011). These tightly bound molluscs create distinct communities that engineer their surrounding environment (Kasoar et al. 2015). The majority (~85%) of the world's shellfish reefs are gone, fallen to overharvesting, habitat loss, pollution, and disease (reviewed by Beck et al. 2011; Gilby et al. 2018; Gillies et al. 2018). In North America, Europe, and Australia, several oyster reef habitats are functionally extinct (Beck et al. 2011; Gillies et al. 2020). Numerous efforts are underway globally to restore shellfish reefs using artificial constructions of recycled shell, crushed concrete, or natural rock (Coen and Luckenbach 2000; Hernández et al. 2018; McAfee et al., 2022).

Many studies that have investigated the efficacy of artificial structures in restoring shellfish reefs measured changes in biodiversity (e.g., Gilby et al. 2019; Xu et al. 2023). The capacity of artificial reefs to restore other ecosystem services, such as water filtration, remains less clear. One of the reasons for this is the difficulty of measuring ecosystem services *in situ*. Current methods to measure the amount of water filtered by organisms (hereafter clearance rates) are limited in size and volume, are not easily replicated, are impractical for manipulative experiments, and vulnerable to tide and weather (Riisgård 2001; Hansen et al. 2011). Some studies have attempted to overcome these limitations by measuring clearance rates by bivalves in the laboratory (e.g., Castle and Waltham 2022; Cottingham et al. 2023) or in the field using devices tailored to specific situations and species (Riisgård 2001; Galimany et al. 2011). However, studies on single species and individuals often overestimate clearance rates by as much as an order of magnitude compared to values obtained for communities (Hansen et al. 2011). A practical method to measure clearance rates of whole communities living on restored oyster reefs is needed.

In this study, we tested the potential for modular shellfish reef restoration baskets to be used to measure clearance rates *ex situ* and for manipulative experiments measuring the robustness of marine communities living on restoration structures through real-world application. We used modular baskets (Fig. 1) to provide baseline data on clearance rates of invertebrate communities colonising shellfish reef restoration structures and then examined how clearance rates were affected by variation in light, temperature, and salinity.

Materials and Procedures

Robust Oyster Basket 400

Robust Oyster Baskets 400 (hereafter ROB 400) are a shellfish reef restoration structure developed by the not-for-profit organisation OzFish Unlimited (Fig. 1). The prism-shaped design (400 × 400 × 300 mm) is made of steel mesh encasing recycled oyster shells (Fig. 1a) which, after deployment, is colonised by mixed communities dominated by filter-feeding bivalves (particularly rock oysters) (Fig. 1b). Ten ROB 400s were collected (July 2023) after 19 months of deployment in Moreton Bay, Queensland, Australia (-27.45684, 153.39528), transported out of water, and housed individually in aerated 110-L polyethylene tanks (Fig. 1c) at the University of Queensland's Moreton Bay Research Station, Minjerribah, Queensland, Australia from July to December 2023. Seawater at ambient temperature (35–37 salinity, ~21 °C) was recirculated among ten tanks containing ROB 400s, six tanks without ROB 400s, and a 400-L sump with mechanical filtration (Fig. 1c). ROB 400s were fed a mixed diet of live *Nannochloropsis oceanica* (CS-246, CSIRO Australian National Algae Culture Collection, Hobart, Tasmania, F media [Cell-hi F2P, Varicon aqua], 25 °C, ~35 salinity, 22:2 h light/dark photoperiod) and Shellfish Diet 1800 (Reed Mariculture) three times per week. Seawater (50–66%) was exchanged monthly. When ROBs were added to the system, ammonia levels spiked to 0.5–1.0 ppm (API Ammonia NH_3/NH_4^+ test kit) but fell below 0.5 ppm within 3 days. Thereafter ammonia was undetectable (<0.25 ppm). Temperature, salinity, and dissolved oxygen (DO) were monitored with a Horiba U-52 Series MultiParameter Water Quality Meter. Values recorded during experiments are reported in Supporting Information Table S1. ROB 400s were left undisturbed for at least 14 days between experiments. The surface area colonised by invertebrates, the number of living and dead filter feeding invertebrates (>10 mm), and the height and length (*sensu* Galtsoff 1964) of 20 haphazardly selected rock oysters (the dominate invertebrates present) were measured using a tape measure, by visual count, and verniers, respectively, 14 days after the final (salinity) experiment (Supporting Information Table S2).

Baseline clearance rates

To quantify baseline clearance rates, we measured changes in the density of living microalgae (*N. oceanica*) in tanks with ROB 400s compared to tanks without ROB 400s. At the beginning of the experiment, water flow was turned off, which created ten independent tanks housing ROB 400s and six independent tanks without ROB 400s. Each tank was continuously aerated to maintain DO and keep microalgae in suspension. Live *N. oceanica* were added to each tank at an initial mean density of 1.3×10^6 cells $mL^{-1} \pm 5.2 \times 10^4$ SD.

Absorbance (sum of λ750, λ664, λ647, λ630) was measured at the beginning of the experiment and every 30 min thereafter until 2 h had elapsed or the density of the microalgae fell below ~9×10^4 cells mL^{-1} (spectrophotometer, Hach DR 5000™ UV-Vis, Starn Pty Ltd glass cuvette, Type 1, match code 7, path length 10 mm). Cuvettes were triple rinsed between samples, and samples were directly pipetted from the respective tank. Data on the density of *N. oceanica* in each tank at each time point were derived from absorbance data (Supporting Information Fig. S1). Clearance rates were then calculated for each replicate per Eq. 1 modified from Riisgård (2001):

$$\boldsymbol{Cl = (V/t)\, ln(C_0/C_t)} \qquad (1)$$

where C_0 and C_t equal the concentration of microalgae (cells mL^{-1}) at the time points zero and t respectively, and V equals the volume of water (Supporting Information Table S2).

Effect of light on clearances rates

There is some evidence marine shellfish can detect and respond to light (Wu et al. 2015), though it is not clear whether feeding is affected by light. To test whether clearance rates by invertebrate communities were different when exposed to light or dark, five ROB 400s tanks and three control tanks without ROB 400s were randomly assigned to a ‘dark’ treatment and covered (top and sides) with black polythene (GRUNT GRGB0042, mean 5.1 lux ± 2.6 SD at the water surface, HOBO MX Temp/Light MX 2202 set to record every 10 min), while the remaining five ROB 400s tanks and three control tanks were assigned to a ‘light’ treatment and left uncovered exposed to constant light (LED ‘cool white’, mean 455.7 lux ± 5.6 SD at the water surface, HOBO MX Temp/Light MX 2202 set to record every 10 min). After 18 h, water flow was turned off and live *N. oceanica* were added to each tank at an initial mean density of 1.09×10^6 cells $mL^{-1} \pm 5.5 \times 10^4$ SD. Absorbance was measured as previously described and used to derive clearance rates for all replicates in the light and dark treatments for the period 0–60 min after microalgae was added.

Effect of temperature on clearance rates

We simulated a marine heatwave lasting a period of 5 days where mean temperatures were ~4 °C above ambient for that time of year (*sensu* Hobday et al. 2016). The heatwave treatment was applied to five randomly allocated tanks containing ROB 400s and three randomly allocated control tanks without ROB 400s that were heated by 3–5 °C using 300 W titanium aquarium heaters (Aqua One TH300 or Aqualogic). Five tanks with ROB 400s and three control tanks without ROB 400s were left at ambient temperatures of ~20 °C. Water flow was turned off when heaters were added to the tanks (time 0) and remained off for the duration of the experiment. Absorbance was measured after 24 and 120 h as previously described and used to derive clearance rates for all replicates in the heatwave and ambient treatments for the period 0–60 min after microalgae was added.

Effect of salinity on clearance rates

To test the effects of a simulated flood, salinity was lowered with tap water (21.5 °C, <0.01 salinity) in randomly allocated tanks to levels recorded in Moreton Bay during the 2010/2011 Brisbane River flood (Oubelkheir et al. 2014; Clementson et al. 2021); ~15 salinity in three tanks containing ROB 400s and three control tanks without ROB 400s, or ~25 salinity in four tanks containing ROB 400s and three control tanks without ROB 400s. Salinity was not altered in three tanks containing ROB 400s (salinity 36.2). AquaOne Water Conditioner© ($Na_2S_2O_3$, H_2O) was added to all tanks (10 mL per tank). Water flow to all tanks was turned off before the freshwater was added (time 0) and remained off for the duration of the experiment. Absorbance was measured after 24, 72, and 120 h as previously described, and used to derive clearance rates for the reduced salinity and ambient treatments for the period 0–120 min after microalgae was added. After 120 h, salinity levels had increased by ~1 in all tanks due to evaporation (Supporting Information Table S1).

Statistical analysis

Data on algal density in the control tanks for the baseline study were analysed using repeated measures ANOVA design in Primer v7 with ‘time’ as a random factor. Replicate (tank) was included in the model to account for non-independence of measurements taken from the same replicate over time. A type I sum of squares was used. Data on clearance rates in the light/dark experiment were analysed by two-way ANOVA in SPSS v29.0 using ‘presence/absence of ROB 400’ and 'treatment’ as fixed factors, and tank as the level of replication. A type III sum of squares was used. Data on clearance rates in the heatwave experiment were analysed using repeated measures ANOVA design in Primer v7 with ‘day’ as a random factor and ‘presence/absence of ROB 400’ and ‘treatment’ as fixed factors, respectively. Replicate (tank) was included in the model to account for non-independence of measurements taken from the same replicate over time. A type I sum of squares was used. For the flood experiment, replicates with ROB 400s and replicates without ROB 400s were analysed separately. Data on clearance rates were analysed using repeated measures ANOVA design in Primer v7 with ‘day’ as a random factor and ‘treatment’ as a fixed factor. Replicate (tank) was included in the model to account for non-independence of measurements taken from the same replicate over time. A type I sum of squares was used.

For all ANOVAs, assumptions of normality and heterogeneity of variance were examined using Q-Q residual plots, values for skewness and kurtosis, and Kolmogorov-Smirnov and Shapiro-Wilk tests in IBM SPSS v29.0 (Quinn and Keough 2002; Field 2018). Data for the simulated flood experiment with ROBS present were not normally distributed, but as analyses done using transformed data gave the same outcomes as analyses of untransformed data, analyses done using untransformed data were presented. All other data met the assumptions of ANOVA. Any outliers were included in analyses as these had little effect on the outcomes. Significant outcomes ($p < .05$) with more than two levels were interrogated by pair-wise (Primer 7) or Bonferroni (SPSS v29) post hoc tests.

Assessment

Baseline clearance rates

Density of *N. oceanica* fell in tanks containing ROB 400s (Fig. 2a), decreasing by almost 85% after 90 min (Fig. 2b). In contrast, the density of *N. oceanica* in control tanks without ROB 400s remained stable (Fig. 2a) and was not significantly different after 90 min compared to the initial density (repeated measures ANOVA, $F_{1,9} = 0.03$, $p = .204$, Fig. 2b). The mean clearance rate of tanks with ROB 400s present was 119.1 L h^{-1} ± 14.8 SE, though clearance rates were not consistent over time (Fig. 2c). For instance, mean clearance rates were initially 70.8 L h^{-1} ± 4.8 SE but more than doubled to 175.4 L h^{-1} ± 33.4 SE in the interval from 60–90 min after microalgae were added (Fig. 2c).

Effect of light on clearance rates

There was no effect of light on the clearance rates of tanks containing ROB 400s (Fig. 3, Table 1). Tanks with ROB 400s present had significantly higher clearance rates than control tanks without ROB 400s (Table 1, present > absent).

Effect of temperature on clearance rates

An increase in temperature (~4 °C) had no effect on clearance rates of the invertebrate communities living on ROB 400s over five days (Fig. 4, Table 1). Tanks with ROB 400s present had significantly higher clearance rates than control tanks without ROB 400s (Table

1; present > absent). Clearance rates did not significantly vary across time (Fig. 4, Table 1), and there were no significant interactions among any factors (Table 1).

Effect of salinity on clearance rates

In tanks containing ROB 400s, salinity had a significant effect on clearance rates (Fig. 5, Table 1). Post hoc pair-wise tests indicated there was an overlapping hierarchy of significance, with clearance rates higher in the 25 salinity treatment than in the 15 salinity treatment, but clearance rates in the ambient treatment were not significantly different than either the 25 and 15 salinity treatments (Table 1; 25 = ambient > ambient = 15). In tanks without ROB 400s, there was no effect of salinity, but clearance rates varied among sampling times (24 = 120 > 120 = 72 h), with no significant interactions among factors (Fig. 5, Table 1).

Discussion

In ambient conditions, mean clearance rates by invertebrate communities on ROB 400s varied between 251.0 and 522.6 L h^{-1} m^{-2} among experiments. These values are similar to clearance rates reported for invertebrate communities dominated by oysters and mussels in field studies (e.g., Hansen et al. 2011; Vismann et al. 2016; Rullens et al. 2022). For instance, bivalve beds dominated by *Crassostrea gigas* and *Mytilus edulis* had clearance rates of 138.6 ± 32.7 L h^{-1} m^{-2} ($n = 18$) and 447.2 ± 97.8 L h^{-1} m^{-2} ($n = 16$) respectively (Vismann et al. 2016). In a mussel-dominated shellfish bed, clearance rates increased from 193.5 to 806.1 L h^{-1} m^{-2} after microalgae was added (Hansen et al. 2011). We also found rock oyster-dominated invertebrate communities living on ROB 400s increased their clearance rates through time, a trend also observed for freshwater rainbow mussels, *Villosa iris*, fed a high food ration (Gatenby et al. 2013). One explanation for this could be compensatory food intake where filter feeders increase the amount of water they pass though their bodies as algae concentrations decrease (Bayne et al. 1987; Barillé et al. 1993; Bayne et al. 1993). Our results demonstrate modular restoration structures can be used to obtain data on clearance rates of whole communities *ex situ* without the inherent difficulties of sampling in the field and over-estimation of clearance rates extrapolated from measurements on individuals (Hansen et al. 2011).

We found no effect of light on clearance rates of invertebrate communities occupying ROB 400s. We are not aware of any other study that has tested the effects of light on a community of marine filter feeders, but studies done on freshwater mussels indicate that the effects of light on clearance rates is species specific (Hills et al. 2020; Pouil et al. 2021). For instance, exposure to darkness led to increases in clearance rates of the Asian clam, *Corbicula fluminea*, but not the paper pondshell, *Utterbackia imbecillis* (Hills et al. 2020). The negligible impact of light in this study might be because communities were dominated by intertidal rock oysters. Intertidal species are generally less affected by light because they must feed while they are submerged to gain adequate nutrition, regardless of the time of day (Loosanoff and Nomejko 1946).

We found no effect of an increase in temperature on clearance rates of invertebrate communities living on ROB 400s. This contrasts with studies performed on single bivalve species which found that clearance rates generally increase with temperature until a thermal limit is reached beyond which feeding is depressed and clearance rates rapidly decline (e.g., Ren et al. 2000; Yukihira et al. 2000; Parker et al. 2024). One explanation for why our results differ from those of previous studies could be that the invertebrate communities we tested were adapted to an intertidal environment where they are regularly exposed to a broad range of temperatures (Potter and Hill 1982; Helmuth et al. 2006). We also simulated a marine heatwave occurring during winter or early spring. An increase in temperature of 4 °C is more likely to influence clearance rates during summer when the rock oysters that dominated the ROB 400s would be closer to their upper thermal limit, especially in scenarios of aerial heatwaves occurring during low tide (Dove and O'Connor 2007; Scanes et al. 2020).

Clearance rates were on average ~45% lower when salinity was ~25 compared to ~15, perhaps because the invertebrates living in the ROB 400s approached their tolerance limit at the lower extremes of salinity these invertebrate communities experience in nature (Oubelkheir et al. 2014; Clementson et al. 2021). Reductions in clearance rates due to exposure to low salinity have been reported for a wide range of invertebrate communities and species (e.g., Navarro and Gonzalez 1998; McFarland et al. 2013; Casas et al. 2018). Our results indicate the invertebrate communities living on the ROB 400s may have been stressed when exposed to very low salinity but seemingly continue to provide substantial filtration services regardless. As clearance rates were greatest at a salinity of ~25, shellfish reef restoration may be as effective in boosting water filtration ecosystem services in estuarine and coastal waterways, where the salinity is usually lower than in the ocean.

Comments and recommendations

The density of microalgae in the control tanks was always not stable, sometimes declining presumably as microalgae fell to the bottom or slightly increasing as microalgae reproduced or clumps of microalgae broke up. Filtering algae cultures prior to use or using a microalga that is neutrally buoyant and/or swims may help to prevent this occurring. The propensity of the density of living microalgae to vary over time highlights the importance of using controls for accurate evaluation of clearance rates. To date, most studies investigating bivalve clearance rates included controls only when performed in a laboratory setting. Filtration studies on invertebrate communities performed *in situ* generally lack controls (e.g., Hansen et al. 2011). We suggest future studies should include controls to avoid overestimating the clearance rates of filter-feeding invertebrate communities.

The ROB 400s used in this study were initially deployed in the intertidal zone and subsequently colonised by organisms adapted to intermittent emersion. This likely explains why these communities appeared to cope well with transport to the laboratory and subsequent experiments. The proportions of deceased shellfish within communities on the ROB 400s at the end of this study are similar to those found on ROB 400s deployed in Moreton Bay (Porter, unpublished data). Likewise, there was only a small spike in ammonia levels in the days after transport, suggesting there was little mortality and decomposition (Canfield et al. 2010). Marine invertebrates living on subtidal modular restoration structures may be less robust when exposed to air compared to the same species living in the intertidal (e.g., Widdows and Shick 1985; Giomi et al. 2016). Future studies should consider the need to transport communities in water to reduce lethal and sublethal impacts that could influence clearance rates in subsequent experiments.

This study demonstrates modular restoration structures can be used to test the effects of physiochemical parameters (e.g., light, temperature, salinity) on clearance rates of invertebrate communities. Modular baskets bypass common challenges that occur during filtration studies on bivalves, both *ex situ* (e.g., high level of disturbance, small volumes, often restricted to few animals and/or single species) and *in situ* (e.g., dependence on weather and tide, inability to manipulate physiochemical parameters, lack of controls, poor replication). Additional studies are recommended to expand our baseline study by, for instance, evaluating the clearance rates of the invertebrate communities in a wider range of experimental conditions predicted over the next century (e.g., reduced pH, reduced oxygen levels, increased turbidity), and to test identical and more extreme conditions over extended periods as extreme events often last beyond the 5 days tested here (e.g., reduced salinity over weeks).

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Acknowledgments

We extend our deepest respect and recognition to all First Nations Peoples of the Quandamooka Country, where this study was conducted, who continue cultural and spiritual connections to Country. We recognise their valuable contributions to Australian and global society. We thank the volunteers and staff at OzFish Unlimited Moreton Bay chapter for their work in creating, deploying, and retrieving the ROBs, with special thanks to Dave Smith, Bill Milligan, Gavin Gray, and Randall Kirkwood for assistance with counting invertebrates. We also thank UQ students Myles Munro and Raileh Linton for assistance with sampling and the kind professional staff at the Moreton Bay Research Station for help with logistics and administration. BM was supported by an Australian Research Council-funded DAATSIA (Discovery Aboriginal and Torres Strait Islander Award, IN2000100026).

Figures

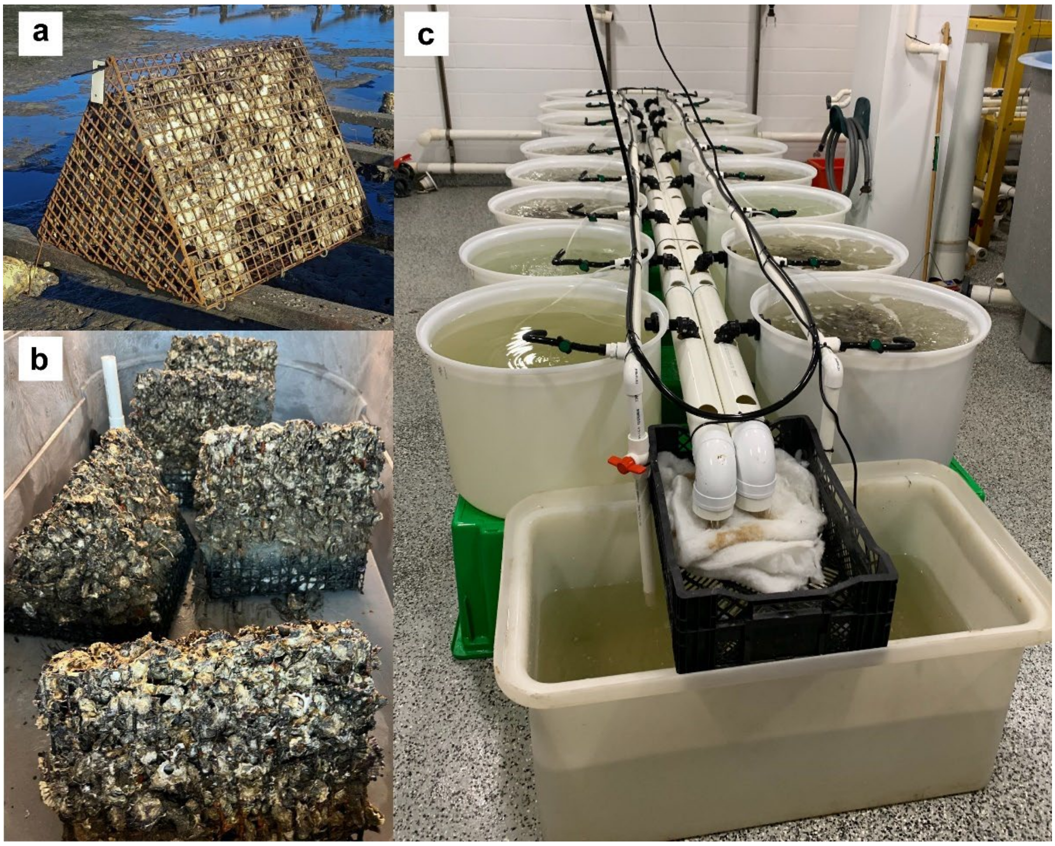

Fig. 1. Modular shellfish reef restoration structures and experimental setup used in this study. **a**. The Robust Oyster Basket (ROB 400) prior to deployment. OzFish Unlimited (n.d.). **b**. ROB 400s colonised by invertebrate communities dominated by rock oysters following 19 months deployment in the intertidal zone in Moreton Bay, Australia. Andersson (2023). **c**. ROB 400s in the experimental setup used to test the effects of light, temperature, and salinity on clearance rates. Mos (2023).

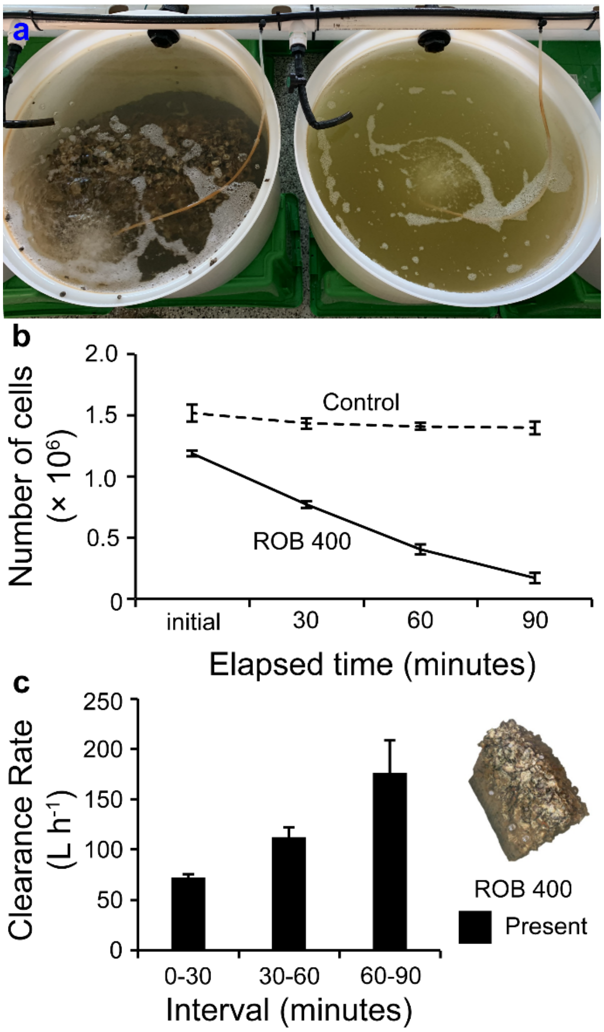

Fig. 2. Clearance of microalgae, *Nannochloropsis oceanica* (CS-246) by marine invertebrate communities living on modular shellfish reef restoration structures (ROB 400). **a**. Illustrative density of *N. oceanica* in tanks with ROB 400s present (left) and absent (right) 2 h after microalgae were added. **b**. Density of *N. oceanica* over 90 min in static, aerated tanks without modular shellfish reef restoration structures (Control, dashed line) and with modular shellfish reef restoration structures (ROB 400, solid line). Data are means ± SE, $n = 9$ for ROB 400, $n = 5$ for control. **c**. Clearance rates of invertebrate communities living on ROB 400s in each 30-minute interval over the 90 min experiment. Data are means ± SE, $n = 9$.

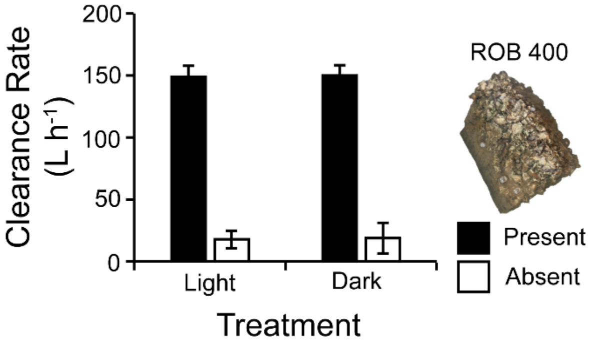

Fig. 3. Effects of light and the presence/absence of modular shellfish reef restoration structures housing rock oyster-dominated invertebrate communities (ROB 400) on clearance rates in static, aerated tanks. Tanks were held in darkness (mean 5.1 lux ± 2.6 SD) or light (mean 455.70 lux ± 5.60 SD) for 18 h before *Nannochloropsis oceanica* CS-246 was added at a density of ~1.1×10^6 cells mL^{-1}. Clearance rates were derived from data on changes in the density of *N. oceanica* after 60 min. Clearance rates were affected by the presence of ROBs (present > absent) but were not influenced by treatment nor the interaction between these factors (Table 1). Data are means ± SE; *n* = 5 for present, *n* = 3 for absent.

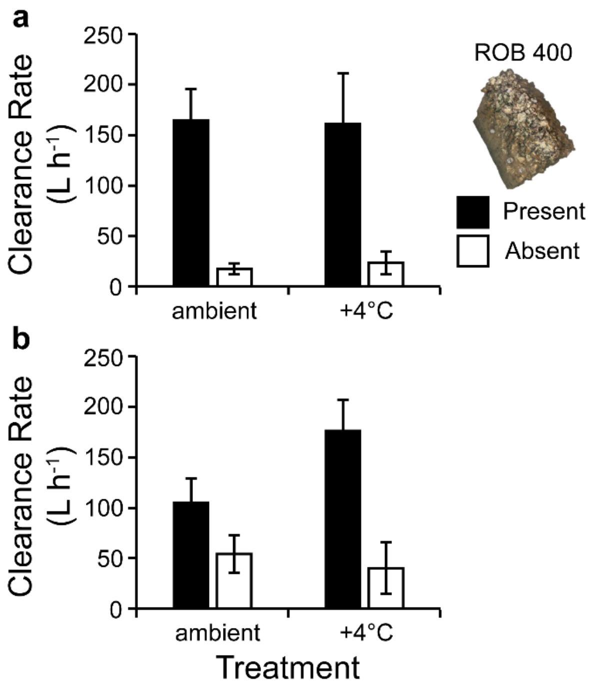

Fig. 4. Effects of temperature and presence/absence of modular reef restoration structures housing rock oyster-dominated invertebrate communities (ROB 400) on clearance rates in static, aerated tanks. Tanks were held at ambient (~21 °C) and warmed (+4 °C, ~25 °C) temperatures for **a**. 24 h and **b**. 120 h before *Nannochloropsis oceanica* CS-246 was added at an initial density of ~1–1.5 × 10^6 cells mL^{-1}. Clearance rates were derived from data on changes in the density of *N. oceanica* after 60 min. Clearance rates were affected by the presence of ROB 400s (present > absent) but were not influenced by temperature, time, nor any interaction among these factors (Table 1). Data are means ± SE; *n* = 3–5 for present, *n* = 3 for absent.

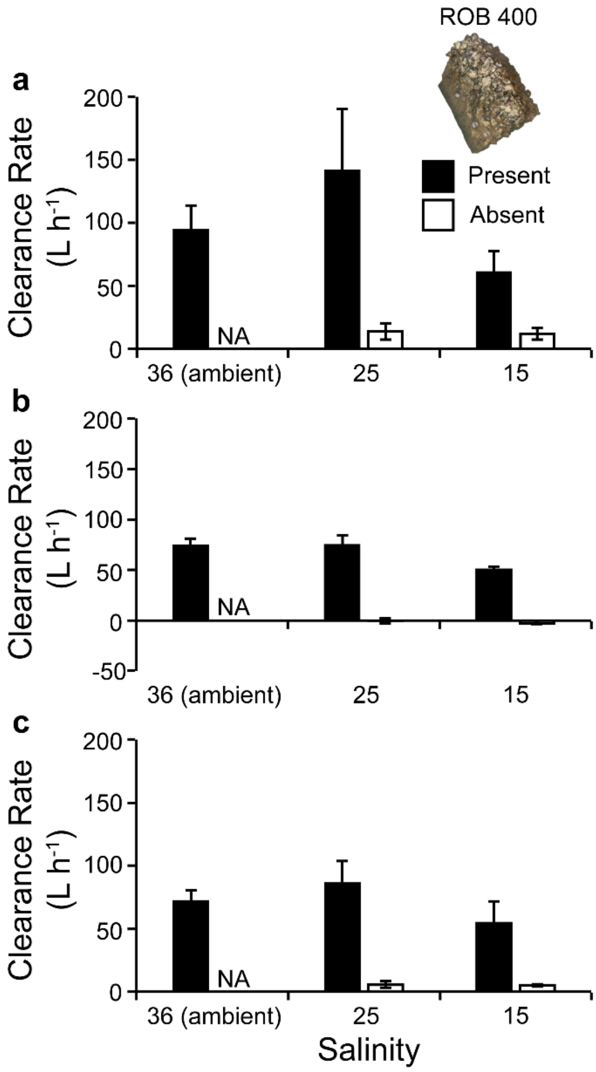

Fig. 5. Effects of salinity and the presence/absence of modular reef restoration structures housing rock oyster-dominated invertebrate communities (ROB 400) on clearance rates in static, aerated tanks. Tanks were held at ambient salinity (~36) or reduced salinity (~25 or ~15) for **a**. 24h, **b**. 72 h, and **c**. 120 h before *Nannochloropsis oceanica* CS-246 was added at a density of ~1.5×10^6 cells mL^{-1}. Clearance rates were derived from data on changes in the density of *N. oceanica* after 120 min. No data for ambient salinity-absent treatment was available (NA). Note different scale for the y-axis in panel **b**. Clearance rates for tanks with (present) and without (absent) ROB 400s were statistically analysed separately (Table 1). When ROB 400s were present, clearance rates were affected by salinity (25 = 35 > 35 = 15) but were not influenced by time nor any interaction between these factors (Table 1). When ROB 400s were absent, clearance rates varied across time (24 h =120 h > 120 h = 72 h) but were not influenced by salinity nor any interaction between these factors (Table 1). Data are means ± SE; *n* = 3 for present except for present-25 where *n* = 4; *n* = 3 for absent.

Tables

Table 1. Outcomes of ANOVA analyses examining the effects of light, temperature, and salinity on the clearance rates of tanks with and without invertebrate communities living on modular shellfish reef restoration structures (ROB 400) in laboratory experiments. df, degrees of freedom; MS, mean square; p/a, presence/absence; temp, temperature. Significant factors are in bold ($p < .05$).

Parameters	Source	df	MS	F	*p*	Post hoc tests
Light	**presence/absence**	**1**	**1.13E5**	**325.38**	**<.0001**	present > absent
	treatment	1	3.70	0.01	.919	
	p/a × treatment	1	2.30E-2	<0.01	.994	
	error	12	347.29			
Temperature	time	1	2.43E3	1.17	.308	
	presence/absence	**1**	**1.16E5**	**18.81**	**<.0005**	present > absent
	temperature	1	3.21E3	0.59	.468	
	p/a × temp	1	4.52E3	0.54	.754	
	p/a × time	1	351.91	0.17	.700	
	temp × time	1	2.89E3	1.38	.277	
	temp × time × p/a	1	6.26E3	3.00	.117	
	replicate (temp × p/a)	12	6.10E3	2.92	.067	
	error	9	2.09E3			
Salinity (Present)	**treatment**	**2**	**5.34E3**	**2.33**	**.040**	25 = 35 > 35 = 15
	time	2	3.82E3	2.09	.118	
	treatment × time	4	938.08	0.51	.797	
	replicate (treatment)	7	2.13E3	1.17	.370	
	error	14	1.82E3			
Salinity (Absent)	treatment	1	11.19	1.34	.348	
	time	**2**	**325.66**	**8.05**	**<.001**	24 h = 120 h > 120 h = 72 h
	treatment × time	2	0.99	0.25	.976	
	replicate (treatment)	4	37.50	0.93	.491	
	error	8	40.44			

Supporting Information for

Quantifying clearance rates of restored shellfish reefs using modular baskets

Maja Andersson[1,2], Karen L. Cheney[3,4], Robbie Porter[5], Ben L. Gilby[6], Benjamin Mos[4,7]

[1] School of the Environment, The University of Queensland, Brisbane, QLD, 4072 Australia

[2] Biology Education Centre, Uppsala University, Norbyvägen 14, 752 36 Uppsala, Sweden

[3] Marine Sensory Ecology Group, School of the Environment, The University of Queensland, Brisbane, QLD, 4072 Australia

[4] Centre for Marine Science, The University of Queensland, Brisbane QLD, 4072 Australia

[5] OzFish Unlimited Ltd, Level 1, 299 River Street, Ballina, NSW, 2470, Australia

[6] School of Science, Technology and Engineering, University of the Sunshine Coast, Petrie, QLD, 4502, Australia

[7] Moreton Bay Research Station, School of the Environment, The University of Queensland, Dunwich/Goompi, QLD, 4183, Australia

Corresponding author: B. Mos

Address: Moreton Bay Research Station, School of the Environment, The University of Queensland, 37 Fraser Street, Dunwich, QLD 4183, Australia

Email: b.mos@uq.edu.au

This file includes:

Tables S1 to S2

Figure S1

Table S1. Mean water parameters (temperature (°C), salinity, and dissolved oxygen (mg L^{-1})) for **a.** temperature and **b.** salinity treatments during laboratory experiments testing clearance rates in tanks containing mixed invertebrate communities living on oyster baskets (ROB 400) and tanks without ROB 400s (Control). Values in parentheses are standard deviation. – data not available

Experiment		**Treatment**	**Elapsed time (h)**	**Temperature**	**Salinity**	**Dissolved oxygen**
a	ROB 400	+4°C	24	24.1 (0.2)	–	–
	Control	+4°C	24	24.7 (0.7)	–	–
	Rob 400	Ambient	24	21.9 (0.1)	–	–
	Control	Ambient	24	21.7 (0.2)	–	–
	ROB 400	+4°C	72	24.1 (0.2)	–	–
	Control	+4°C	72	24.1 (0.8)	–	–
	ROB 400	Ambient	72	19.5 (0.2)	–	–
	Control	Ambient	72	19.4 (0.1)	–	–
	ROB 400	+4°C	120	24.0 (0.2)	39.5 (0.1)	4.8 (0.3)
	Control	+4°C	120	24.3 (0.8)	38.9 (0.2)	4.7 (0.2)
	ROB 400	Ambient	120	19.3 (0.1)	38.3 (0.2)	6.9 (0.5)
	Control	Ambient	120	19.1 (0.3)	37.8 (0.3)	6.9 (0.8)
b	ROB 400	15 salinity	24	21.0 (0.1)	15.5 (0.5)	6.9 (0.2)
	Control	15 salinity	24	21.0 (0.1)	15.1 (0.1)	7.2 (0.3)
	ROB 400	25 salinity	24	20.9 (0.1)	25.6 (0.2)	6.0 (0.2)
	Control	25 salinity	24	21.0 (0.1)	24.7 (0.2)	7.1 (0.1)
	ROB 400	36 salinity	24	21.0 (0.1)	35.7 (0.3)	6.0 (0.1)
	ROB 400	15 salinity	72	21.1 (0.1)	15.8 (0.1)	7.1 (0.4)
	Control	15 salinity	72	20.8 (0.1)	15.7 (0.1)	8.0 (0.7)
	ROB 400	25 salinity	72	21.0 (0.1)	26.0 (0.2)	6.2 (0.3)
	Control	25 salinity	72	21.0 (0.1)	24.8 (0.1)	6.6 (0.4)
	ROB 400	36 salinity	72	21.0 (0.1)	36.4 (0.1)	6.3 (0.3)
	ROB 400	15 salinity	120	21.5 (0.1)	16.7 (0.1)	7.3 (0.2)
	Control	15 salinity	120	21.2 (0.1)	16.1 (0.2)	7.8 (0.2)
	ROB 400	25 salinity	120	21.4 (0.1)	26.1 (0.3)	7.1 (0.3)
	Control	25 salinity	120	21.4 (0.1)	25.3 (0.2)	6.5 (1.1)
	ROB 400	36 salinity	120	21.3 (0.1)	36.8 (0.1)	5.9 (0.2)

Table S2. Information on the tank setup and robust oyster baskets (ROB 400) used in experiments; the volume of seawater in each tank (L), the surface area of each ROB colonised by filter-feeding invertebrates (m^2), the number of living and non-living (in parentheses) individuals (>1 cm) of the dominate filter-feeders found on the ROB400s, the total number of filter feeders (>1 cm) on each ROB, the height and length (mean ± SD) of 20 rock oysters haphazardly measured from each ROB400, and the treatment randomly allocated to each tank for the three experiments (light/dark, heatwave, and flood).

Tank #		**Volume of seawater in tank (L)**	**Surface Area m^2**	**rock oysters *Saccostrea* spp.**	**hairy mussel *Trichomya cf. hirsuta***	**pearl oyster *Pinctada* cf. *albina***	**total number of filter feeders**	**alive (%)**	**height mm**	**length mm**	**Light/dark**	**Heatwave**	**Flood**
1	Control	107									Light	Ambient	15
2	Control	107									Dark	Ambient	25
3	ROB 400	81.5	0.246	93 (30)	1 (0)	0 (5)	94 (35)	72.9	26.8 (6.9)	32.7 (8.7)	Dark	Ambient	15
4	ROB 400	80	0.259	102 (55)	3 (0)	8 (5)	113 (60)	65.3	26.5 (5.4)	21.8 (6.0)	Light	Heated	36
5	ROB 400	80.5	0.285	121 (43)	0 (0)	5 (8)	126 (51)	71.2	23.8 (9.2)	32.7 (6.9)	Dark	Heated	36
6	ROB 400	80	0.289	170 (144)	7 (1)	3 (3)	180 (148)	54.9	26.0 (9.0)	21.4 (5.4)	Dark	Ambient	25
7	Control	107									Light	Heated	25
8	ROB 400	80	0.299	122 (65)	65 (0)	18 (4)	205 (69)	74.8	21.8 (6.9)	36.0 (8.60)	Light	Heated	25
9	ROB 400	82	0.264	104 (80)	36 (0)	7 (0)	147 (80)	64.8	29.9 (9.6)	21.7 (7.6)	Dark	Ambient	25
10	Control	107									Dark	Heated	15
11	Control	107									Light	Ambient	15
12	ROB 400	83	0.315	157 (138)	25 (1)	8 (7)	190 (146)	56.5	24.5 (6.1)	32.9 (3.2)	Light	Heated	36
13	ROB 400	85.5	0.310	84 (53)	2 (0)	3 (3)	89 (56)	61.4	29.2 (8.4)	25.6 (9.5)	Light	Ambient	25
14	Control	107									Dark	Heated	25
15	ROB 400	81	0.309	169 (173)	22 (0)	2 (10)	193 (183)	51.5	26.6 (7.4)	32.0 (6.6)	Dark	Heated	15
16	ROB 400	81	0.298	172 (103)	7 (1)	0 (4)	179 (108)	62.4	31.5 (9.5)	25.4 (8.3)	Light	Ambient	15
		MEAN	0.287	129.4	16.8	5.4	151.6	63.6					
		±SE	0.024	10.9	6.6	1.7	43.5	2.5					

The mean total surface area colonised by invertebrates on each ROB did not differ among treatments for the light/dark (ANOVA, $F_{1,8} = 1.44$, $p = .242$), heatwave ($F_{1,8} = 0.61$, $p = .458$), or flood ($F_{2,7} = 0.05$, $p = .925$) experiments.

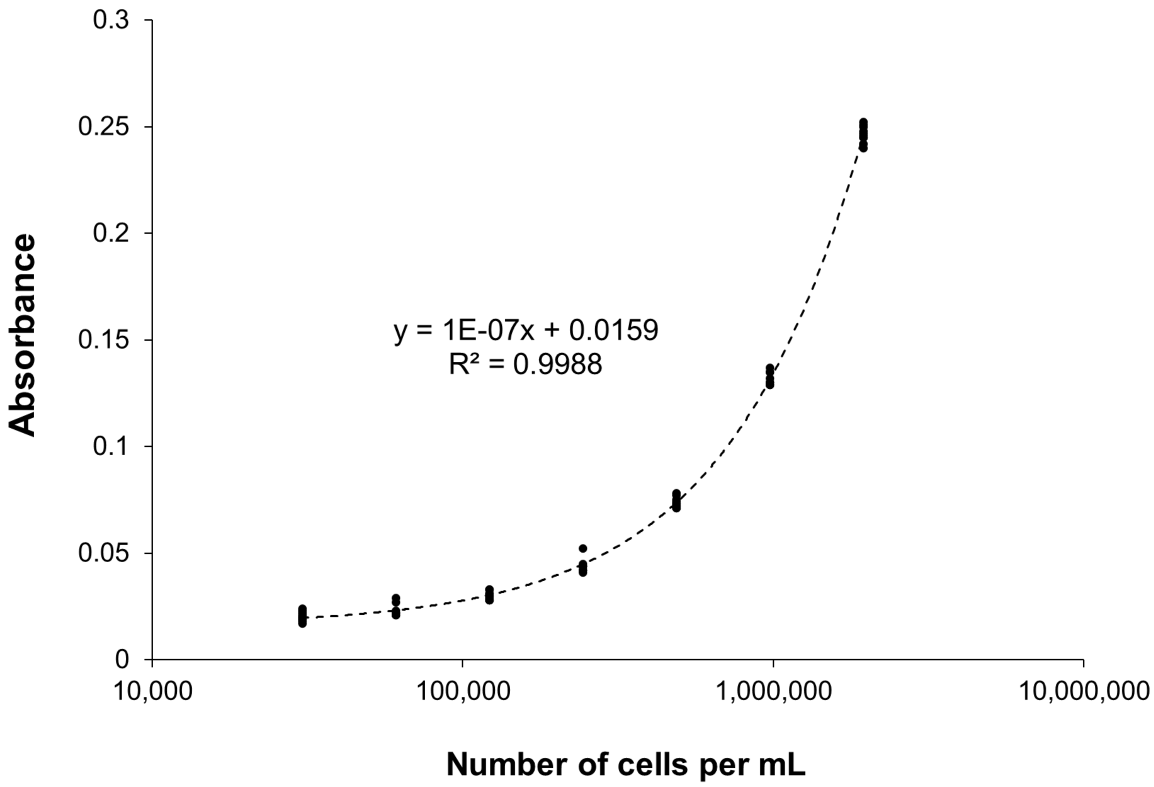

Fig. S1. Relationship between the number of cells per mL of *Nannochloropsis oceanica* (log scale) and total absorbance (sum of λ750, λ664, λ647, λ630) measured using a spectrophotometer (Hach DR 5000™ UV-Vis, Starn Pty Ltd glass cuvette, Type 1, match code 7, path length 10 mm).